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Analysis of the Inhibitory Elements in the p5 Peptide Fragment of the CDK5 Activator, p35, CDKR1 Protein

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Abstract. Besides the hallmark pathology of amyloid plaques and neurofibrillary tangles, it is well documented that cyclin-dependent kinase 5 (CDK5), a critical neuronal protein kinase in nervous system development, function, and survival, when deregulated and hyperactivated induces Alzheimer's disease (AD) and amyotrophic lateral sclerosis and Parkinson's disease-like phenotypes in mice. In a recent study, we demonstrated that p5, a small, truncated fragment of 24 amino acid residues derived from the CDK5 activator protein 35 (NCK5A, p35), selectively inhibited deregulated CDK5 hyperactivity and ameliorated AD phenotypes in model mice. In this study, we identified the most inhibitory elements in the p5 peptide fragment. Each amino acid residue in p5 was systematically replaced with its homologous residues that may still be able to functionally substitute. The effects of these p5 peptide analogs were studied on the phosphotransferase activities of CDK5/p35, CDK5/p25, ERK1, and GSK3β. The mimetic p5 peptide (A/V substitution at the C-terminus of the peptide) in the sequence, KNAFYERALSIINLMTSKMVQINV (p5-MT) was the most effective inhibitor of CDK5 kinase activity of 79 tested mimetic peptides including the original p5 peptide, KEAFWDRCLSVINLMSSKMLQINA (p5-WT). Replacement of the residues in C-terminus end of the peptide affected CDK5 phosphotransferase activity most significantly. These peptides were strong inhibitors of CDK5, but not the related proline-directed kinases, ERK1 and GSK3β.

Keywords: Alzheimer's disease, CDK5 activator protein 35, cyclin-dependent kinase 5, phosphorylation

INTRODUCTION

Phosphorylation of neuronal cytoskeletal proteins is topographically and stably regulated during nervous system development and function. Although protein kinases substrates and regulators are synthesized in the soma, phosphorylation of cytoskeletal proteins such as neurofilaments is consigned to axons [1–4]. While studying the protein kinases involved in compartment-specific phosphorylation in neurons, we identified the cell cycle-like kinase, cyclin-dependent kinase 5 (CDK5), as a major kinase responsible for the phosphorylation of proline- directed Ser/Thr repeats in the C- terminus tail domains of human neurofilament proteins [5]. CDK5 is unique among the CDK family of protein kinases; its activity is primarily restricted to neuronal cells due to its neuron specific activators CDKR1 (also known as p35) and CDKR2 (also known as p39). CDK5 is a multifunctional kinase that targets more than a hundred proteins including other protein kinases and phosphatases essential to neuronal development, function, and survival [2, 6–8].

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In recent years, we and others have shown that CDK5 is deregulated and hyperactivated in the brains of patients expressing several neurodegenerative disorders such as Alzheimer's disease (AD) and amyotrophic lateral sclerosis (ALS) [9–13]. A hypothesis has been proposed that CDK5 deregulation arises in stressed neurons (oxidative, amyloid-β, glutamate excitotoxic, or inflammatory), accompanied by increase in Ca^{+2} influx, calpain activation followed by proteolytic cleavage of the p35 activator into a p10 N-terminal fragment and a p25 hyperactivator that stably binds and hyperactivates CDK5 in a CDK5/p25 complex [13–17]. Such complexes have been detected in AD brains, and they may contribute, in part, to the formation of the hyperphosphorylated neurofilament and tau tangles, and the appearance of amyloid plaques and neuronal apoptosis, all of which are hallmarks of AD pathology. Accordingly, CDK5/p25 has been identified as a potential therapeutic target for AD and other neurodegenerative disorders that share a similar pattern of CDK5 hyperactivation [17].

Currently, most therapeutic approaches that target the deregulated CDK5/p25 complex have focused primarily on drugs like Roscovitine that inhibit by interfering with the ATP binding domain of CDK5 [18–20]. These drugs, however, lack specificity, since all kinases including cell cycle CDKs, are vulnerable at the ATP binding site. During the course of our studies on the basis of CDK5/p25 crystal structure, the amino acid residues interacting between CDK5 and p25 chains within 3.5 Angstroms were identified (unpublished data). This analysis identified two peptides derived as truncations of the p35 regulator, a larger 126 amino acid fragment (CIP) and a shorter 24 amino acid peptide (p5). *In vitro*, these peptides inhibited CDK5/p35 and CDK5/p25, respectively, whereas in rodent cortical neurons, only the deregulated CDK5/p25 was specifically inhibited without affecting the endogenous CDK5/p35 activity [21]. We considered these peptides as potential therapeutic candidates for rescuing neurodegenerative disorders in model mice that share the hyperactivated CDK5-induced phenotypes.

In a recent study we demonstrated p5 has a higher inhibitory activity compared to CIP. In the present study, to further understand p5's inhibitory role, we undertook the synthesis of analogues of the parent peptide p5-WT (KEAFWDRCLSVINLMSSKMLQINA) in which each amino acid was individually replaced with homologous residues that may still be able to functionally substitute. This analysis generated 78 mimetic peptides. The effects of these peptides on recombinant human CDK5/p25 phosphotransferase activity were evaluated. In addition to CDK5/p25, the actions of these p5 peptide analogs on the phosphotransferase activities of CDK5/p35, ERK1, and GSK3β were also measured. From these studies, we identified a mimetic p5 peptide, KNAFYERALSIINLMTSKMVQINV (p5-MT), that may feature sufficiently distinct epitopes such that it would not be recognized by antibodies that would react with endogenous p35 and its proteolytic fragments. In addition, p5-MT showed more potent inhibitory activity toward CDK5 compared to p5-WT.

MATERIALS AND METHODS

Quality control and reagents

The various recombinant protein kinase targets employed in the target profiling process were sourced from Signal Chem Pharmaceuticals, Inc. (Richmond, BC, Canada). Quality control testing was routinely performed on each of the protein kinase targets to ensure compliance to acceptable standards. [γ–^{33}P]ATP was purchased from PerkinElmer.

Both enzymes (CDK5/p25 and CDK5/p35) were co-expressed together by baculovirus in Sf9 insect cells using an N-terminal GST tag and purified. Since they were co-expressed together, they were added to the reaction mixture as CDK5/p25 and CDK5/p35 complexes at the same time. The assay conditions for protein kinases were optimized to yield acceptable enzymatic activity. In addition, the assays were optimized to give a high signal-to-noise ratio.

Protein kinase assays

A radioisotope assay format was used for profiling an evaluation of the kinase under investigation and all assays were performed in a designated radioactive working area. Protein kinase assays were carried out at 30°C for 20 min in a final volume of 25 μl according to the following assay reaction recipe:

Set 1:

Component 1. 5 μl of diluted active CDK5/p35 and CDK5/p25 (final assay concentrations of 14 and 15 nM, respectively).

Component 2. 5 μl of assay solution of calf thymus histone H1 (final assay concentration 1 mg/ml).

Component 3. 5 μl of kinase assay buffer (concentrations: 25 mM MOPS, pH 7.2, 12.5 mM β-glycerophosphate, 25 mM $MgCl_2$, 5 mM EGTA, 2 mM EDTA and 0.25 mM dithiothreitol).

Component 4. 5 μl of [γ–^{33}P]ATP (250 μM stock solution, 0.8 μCi).

Component 5. 5 μl of p5 (p5-WT) or p5-MT (p5 A/V) peptide.

Set 2:

Components 1–4 were same as in Set 1.

Component 5. 5 μl of peptide inhibitor (various concentrations) or 10% DMSO.

Each assay was initiated by the addition of [γ–^{33}P] and, after 20 min incubation, was terminated by spotting 10 μl of the reaction mixture onto Multi screen phosphocellulose P81 plate. The Multi screen phosphocellulose P81 plate was washed 3 times for approximately 15 min each time in a 1% phosphoric acid solution. The radioactivity on the P81 plate was counted in the presence of scintillation fluid in a Trilux scintillation counter. Blank control was set up which included all the assay components except the addition of the appropriate substrate (replaced with equal volume of assay dilution buffer). The corrected activity for protein kinase was determined by removing the blank control value. Each experiment was repeated twice in duplicate.

All other materials were of standard laboratory grade. The 79 peptides analogs were produced by SPOT synthesis as large spots and were based on a selected substitution analysis based on the original sequence KEAFWDRCLSVINLMSSKMLQINA and shown in Supplementary Table 1.

RESULTS AND DISCUSSION

By systematic alteration of each amino acid residue in the parent peptide sequence with a homologous amino acid residue, it should be possible to identify the most crucial amino acid positions. It may be feasible to even identify amino acids that may improve the binding of p5 analogues to CDK5. These are mimetic substitutions that preserve and may improve upon the inhibitory activity of the p5 peptide toward CDK5.

Furthermore, the resulting analogues may also be immunologically distinct, which could foster their differentiation from endogenous p35 and its derivatives with antibodies. The actual sequence of p5 cannot be used to create an immunizing peptide that can distinguish between p5 and a shorter peptide with the same sequence. By identifying the most critical amino acids for inhibition, a modified inhibitory peptide may be created to elicit antibodies that could be used to track its distribution in the brain in future immunohistochemistry experiments.

SPOT synthesis of p5 peptide analogues was undertaken as the method of choice for this purpose as it would be the most cost-effective way to identify the critical amino acid residues in p5 that permit it to compete with p25 and/or p35 and inhibit CDK5 more effectively. As shown in Supplementary Tables 1 and 2, using the 78 mimetic peptides of p5, our studies revealed that the most critical amino acid residues for the inhibitory effect of p5 on CDK5/p25 were located in the last four amino acids in the p5 parent peptide (QINA). Replacement of A to V and M (A/V, M) provided for the most effective inhibitory residues substitutions. Conversely, substitution of Q to N, E, or D were the most stimulatory replacements, increasing activity by almost 80%. Equivalent stimulation is seen in the replacement of I to G, and N to G, E, or D.

The initial aim of these experiments was to determine which of the various peptides tested exhibited reduced inhibitory activity so as to identify the most critical amino acids for the interaction. It was also interesting to define replacement amino acids that could inhibit the phosphotransferase activity of CDK5/p25 better than the original peptide p5-WT (sequence KEAFWDRCLSVINLMSSKMLQINA; Peptide-1).

The phosphotransferase activity of CKD5/p25 protein kinase in the presence of each of the 79 peptides listed in Table 1 was assayed by employing the standardized radioactive assay methodology as described in Materials and Methods. The results observed as activity (cpm) are presented in Supplementary Tables 1 and 2. The intra-assay variability was determined to be less than 10%.

The profiling data for the 79 inhibitory peptides assayed against CDK5/p25 in Table 1 are arranged based on lowest to highest counts per minute (cpm). The % Activity and % Change from Control (CFC) values were determined using the original Peptide-1 (sequence KEAFWDRCLSVINLMSSKMLQINA) as the control (100%) and measuring all other counts relative to this. With this method, only four peptides were identified that yielded lower counts against CDK5/p25 than the original peptide sequence. Peptides-77 and 78 showed the most inhibition of CDK5/p25 compared to the control p5-WT peptide, at −26 and −25%, respectively. Peptides-15 and 76 also showed moderate reductions of −8 and −7%. Most of the other peptides gave higher counts (cpm) ranging from +0 to +79% greater than observed with the parent p5 peptide with the histone H1 substrate. The four peptides that gave the highest counts were Peptides-26, 62, 64, and 70. The altered amino acids

Table 1
% Activity of CDK5/p25 in the presence of inhibitory peptides using radiometric assay method arranged from lowest to highest counts (cpm), with two independent experiments in duplicates. Each assay solution contained 10% DMSO; The blank control without histone was 1,217 cpm, and with histone was 202,940 cpm. The cpm values given in the table was subtracted for the blank with histone in the presence of different peptides. The cpm value in the presence of the p5-WT peptide (#1) was taken as 100% phosphotransferase activity. The negative numbers in the percent change from control (%CFC) values correspond to even greater inhibition and positive numbers are less inhibition in the CDK5 phosphotransferase activity relative to the p5- WT peptide

Peptide ID	Peptide Sequence	CPM	% Activity	% CFC
78	KEAFWDRCLSVINLMSSKMLQINM	89762	74	–26
77	KEAFWDRCLSVINLMSSKMLQINV	90305	75	–25
15	KEAFYDRCLSVINLMSSKMLQINA	111665	92	–8
76	KEAFWDRCLSVINLMSSKMLQINL	113022	93	–7
1	KEAFWDRCLSVINLMSSKMLQINA	121167	100	0
20	KEAFWDRALSVINLMSSKMLQINA	121639	100	0
4	KNAFWDRCLSVINLMSSKMLQINA	122973	101	1
33	KEAFWDRCLSIINLMSSKMLQINA	123757	102	2
48	KEAFWDRCLSVINLMTSKMLQINA	126451	104	4
79	KEAFWDRCLSVINLMSSKMLQINS	131811	109	9
16	KEAFWERCLSVINLMSSKMLQINA	135248	112	12
75	KEAFWDRCLSVINLMSSKMLQINI	138095	114	14
14	KEAFFDRCLSVINLMSSKMLQINA	141068	116	16
29	KEAFWDRCLMVINLMSSKMLQINA	141207	117	17
28	KEAFWDRCLYVINLMSSKMLQINA	141312	117	17
61	KEAFWDRCLSVINLMSSKMVQINA	141773	117	17
41	KEAFWDRCLSVINIMSSKMLQINA	142416	118	18
42	KEAFWDRCLSVINVMSSKMLQINA	145310	120	20
3	KDAFWDRCLSVINLMSSKMLQINA	145721	120	20
18	KEAFWNRCLSVINLMSSKMLQINA	149428	123	23
59	KEAFWDRCLSVINLMSSKCLQINA	150317	124	24
17	KEAFWQRCLSVINLMSSKMLQINA	151097	125	25
34	KEAFWDRCLSVLNLMSSKMLQINA	151937	125	25
13	KEAWWDRCLSVINLMSSKMLQINA	152154	126	26
53	KEAFWDRCLSVINLMSYKMLQINA	152906	126	26
38	KEAFWDRCLSVIQLMSSKMLQINA	154117	127	27
50	KEAFWDRCLSVINLMASKMLQINA	154975	128	28
2	REAFWDRCLSVINLMSSKMLQINA	155431	128	28
25	KEAFWDRCGSVINLMSSKMLQINA	155679	128	28
56	KEAFWDRCLSVINLMSSRMLQINA	155892	129	29
49	KEAFWDRCLSVINLMYSKMLQINA	156497	129	29
67	KEAFWDRCLSVINLMSSKMLQLNA	156827	129	29
11	KESFWDRCLSVINLMSSKMLQINA	163262	135	35
37	KEAFWDRCLSVGNLMSSKMLQINA	164154	135	35
22	KEAFWDRCISVINLMSSKMLQINA	165242	136	36
19	KEAFWDKCLSVINLMSSKMLQINA	166245	137	37
8	KEIFWDRCLSVINLMSSKMLQINA	166249	137	37
63	KEAFWDRCLSVINLMSSKMGQINA	166937	138	38
74	KEAFWDRCLSVINLMSSKMLQING	167174	138	38
5	KQAFWDRCLSVINLMSSKMLQINA	167318	138	38
68	KEAFWDRCLSVINLMSSKMLQVNA	167911	139	39
10	KEMFWDRCLSVINLMSSKMLQINA	168848	139	39
58	KEAFWDRCLSVINLMSSKALQINA	170159	140	40
55	KEAFWDRCLSVINLMSMKMLQINA	170786	141	41
12	KEAYWDRCLSVINLMSSKMLQINA	171316	141	41
24	KEAFWDRCASVINLMSSKMLQINA	171581	142	42
43	KEAFWDRCLSVINAMSSKMLQINA	172298	142	42
51	KEAFWDRCLSVINLMMSKMLQINA	174387	144	44
57	KEAFWDRCLSVINLMSSKSLQINA	175515	145	45
9	KEVFWDRCLSVINLMSSKMLQINA	175785	145	45
44	KEAFWDRCLSVINGMSSKMLQINA	176026	145	45
69	KEAFWDRCLSVINLMSSKMLQANA	178232	147	47
72	KEAFWDRCLSVINLMSSKMLQIQA	181214	150	50
23	KEAFWDRCVSVINLMSSKMLQINA	181448	150	50
45	KEAFWDRCLSVINLSSSKMLQINA	182017	150	50

(Continued)

Table 1
(Continued)

30	KEAFWDRCLSAINLMSSKMLQINA	184482	152	52
21	KEAFWDRMLSVINLMSSKMLQINA	185771	153	53
36	KEAFWDRCLSVANLMSSKMLQINA	186090	154	54
31	KEAFWDRCLSGINLMSSKMLQINA	186098	154	54
6	KEGFWDRCLSVINLMSSKMLQINA	186614	154	54
27	KEAFWDRCLTVINLMSSKMLQINA	189548	156	56
7	KELFWDRCLSVINLMSSKMLQINA	190811	157	57
47	KEAFWDRCLSVINLCSSKMLQINA	191850	158	58
35	KEAFWDRCLSVVNLMSSKMLQINA	192606	159	59
46	KEAFWDRCLSVINLASSKMLQINA	193507	160	60
65	KEAFWDRCLSVINLMSSKMLEINA	194286	160	60
54	KEAFWDRCLSVINLMSAKMLQINA	194650	161	61
52	KEAFWDRCLSVINLMSTKMLQINA	195577	161	61
60	KEAFWDRCLSVINLMSSKMIQINA	196418	162	62
39	KEAFWDRCLSVIELMSSKMLQINA	196444	162	62
73	KEAFWDRCLSVINLMSSKMLQIEA	197176	163	63
32	KEAFWDRCLSLINLMSSKMLQINA	197903	163	63
40	KEAFWDRCLSVIDLMSSKMLQINA	198851	164	64
71	KEAFWDRCLSVINLMSSKMLQIDA	199652	165	65
66	KEAFWDRCLSVINLMSSKMLDINA	199850	165	65
62	KEAFWDRCLSVINLMSSKMAQINA	203269	168	68
26	KEAFWDRCLAVINLMSSKMLQINA	204497	169	69
70	KEAFWDRCLSVINLMSSKMLQGNA	207194	171	71
64	KEAFWDRCLSVINLMSSKMLNINA	217093	179	79

residues in these particular peptides were apparently the most critical for the CDK5 inhibitory activity of p5.

Figure 1 summarizes the findings from analysis of the inhibitory actions of the p5-WT mimetic peptide analogs on the CDK5 phosphotransferase activity. We conclude that the most critical amino acid residues for the inhibitory effect of p25 fragment on CDK5 phosphotransferase activity in the presence of p25 activator appear to be: K1, A3, F4, R7, L9, S10, I12, N13, M15, S17, K18, M19, Q21, I22, and N23. These amino acid residues were distributed across the entire sequence, but region from residues 10 to 23 was the most essential for binding, whereas the last amino acid residue at Position 24 could be altered to actually improve the inhibitory activity of p5 peptide analogs. The most effective replacements of A at Position 24 were with the M and V amino acid residues (A24M and A24V). Most of the other amino acid residues replacements of the p5-WT sequence reduced the inhibitory effect of the peptide mimetics on p25-activated CDK5 phosphotransferase activity. The most marked losses of inhibitory activity were observed with the peptides with the S10A, N13D, L20A Q21N, I22G and N23D replacements.

The above experiments provided evidence that mimetic p5 (A/V) corresponding to Peptide-77 in Table 1 with the sequence KNAFYERALSIINLMTSKMVQINV (p5-MT) was one of the most effective inhibitors of CDK5 phosphotransferase activity of the 79 tested peptides. We next carried out a comparative study using the original wild-type p5 peptide corresponding to Peptide-1 in Table 1 with the sequence KEAFWDRCLSVINLMSSKMLQINA (p5-WT) and the p5-MT peptide to ascertain their inhibitory effects on CDK5/p35, CDK5/p25, and two more related proline-directed protein-serine/threonine kinases, GSK3β and ERK1. The results observed as % change of phosphotransferase activity at different inhibitor concentrations are presented in Table 2, while Supplementary Tables 3–6 and 7–10 contains all the raw data for these protein kinase assays with the p5-WT and p5-MT peptides, respectively. The intra-assay variability was determined to be less than 10%. Inhibition of target phosphotransferase activity by the compound gave negative values, while activation of phosphotransferase target activity gave positive values. Only values of >25% change were considered to be significant.

The profiling study for the parent peptide p5-WT against CDK5/p25 showed moderate to strong inhibition of CDK5/p25 phosphotransferase activity with increasing compound concentration (Supplementary Table 3). At 250 μM concentration of this peptide, the CDK5/p25 phosphotransferase activity was inhibited by 59% compared to control. An IC_{50} value of 169 μM was generated (using a graph of log inhibitor versus normalized response with variable slope with the Prism software) for this peptide against CDK5/p25 (Fig. 2).

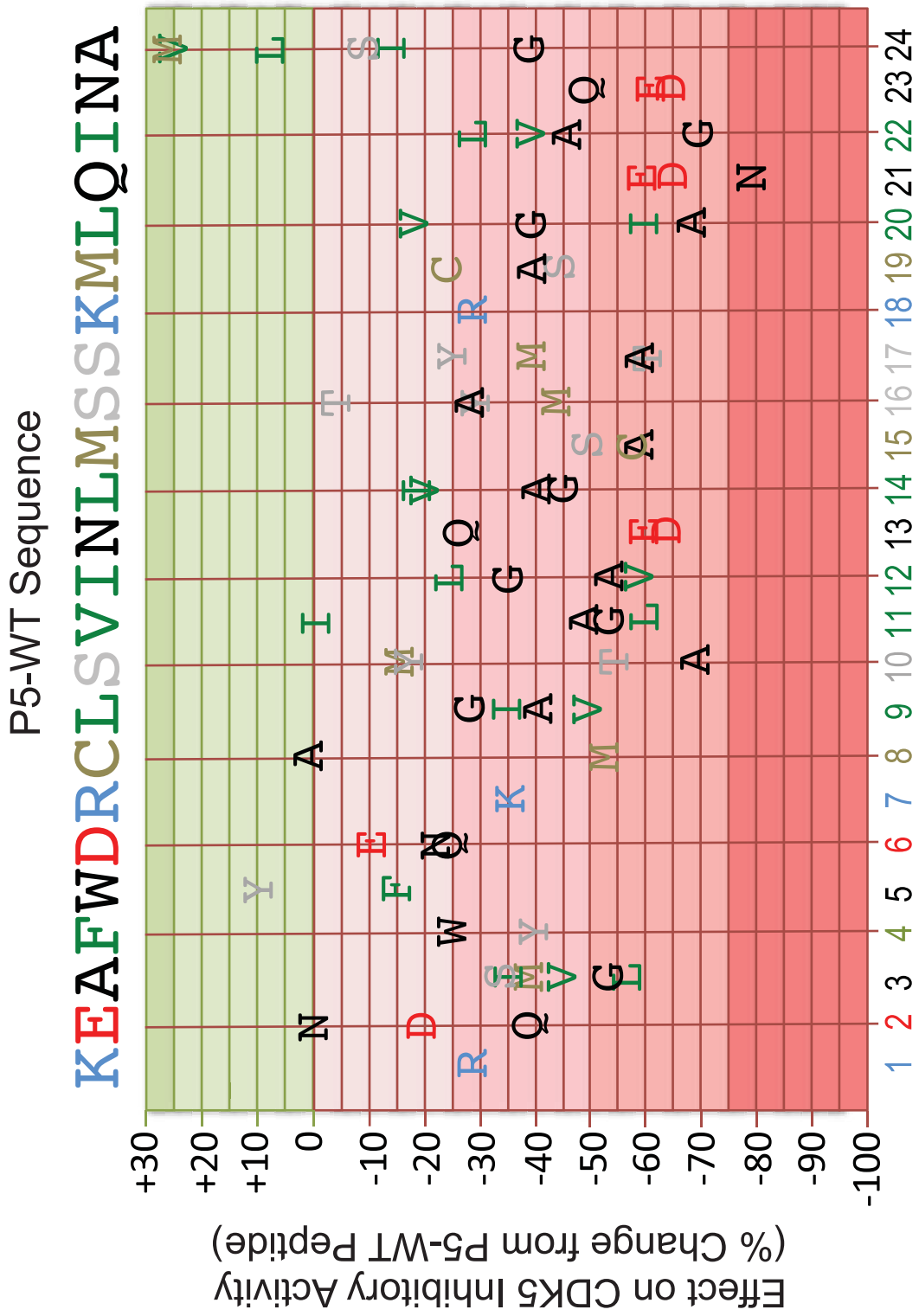

Fig. 1. Mutational analysis of the p5-WT peptide to define the critical amino acids for its inhibitory action on CDK5 phosphotransferase activity. Replacement amino acids are graphed according to their actions on CDK5 kinase activity as a percentage of the effect of the parent p5-WT peptide, which served as a control. Single letter designations are used for each amino acid and these are colored in part according to their charge and hydrophobicity. The corresponding amino acid residue positions are shown in the bottom X-axis.

Table 2
Comparison of the p5-WT and p5-MT peptides for their inhibitory activities toward diverse proline-directed protein kinases. CDK5/p35, CDK5/p25, and two more related proline-directed protein-serine/threonine kinases, GSK3β and ERK1, were tested in duplicate at six different concentrations of these peptides (1 to 500 μM) using a radiometric assay method to determine their IC_{50} values

A. Peptide p5-WT						
Target ID	% Activity Change 1 μM	% Activity Change 10 μM	% Activity Change 50 μM	% Activity Change 100 μM	% Activity Change 250 μM	% Activity Change 500 μM
CDK5/p25	0	–13	–20	–41	–59	–72
CDK5/p35	2	–2	–10	–29	–53	–76
ERK1	0	0	–3	–6	–11	–25
GSK3β	–3	–1	0	8	19	24
B. Peptide p5-MT						
Target ID	% Activity Change 1 μM	% Activity Change 10 μM	% Activity Change 50 μM	% Activity Change 100 μM	% Activity Change 250 μM	% Activity Change 500 μM
CDK5/p25	2	–8	–22	–46	–73	–85
CDK5/p35	–3	–14	–35	–57	–73	–84
ERK1	1	0	–2	–15	–35	–54
GSK3β	–1	3	6	15	27	40

Likewise, the profiling data for p5-WT against CDK5/p35 showed moderate to strong inhibition of CDK5/p35 activity with increasing compound concentration (Supplementary Table 4). At 250 μM concentration of this peptide, the CDK5/p35 activity was inhibited by 53% compared to control. An IC_{50} value of 217 μM was generated (using a graph of log inhibitor versus normalized response with variable slope with the Prism software) for this compound against CDK5/p35 (Fig. 2). These findings reveal that p5-WT inhibited both p25 and p35 activation of CDK5 fairly equally.

The profiling data for compound p5-WT against ERK1 showed much weaker inhibition of ERK1 phosphotransferase activity with increasing compound concentration (Fig. 2). At 500 μM concentration of p5-WT, the ERK1 activity was inhibited by only 25% compared to control. An IC_{50} value of 1322 μM was generated (using a graph of log inhibitor versus normalized response with variable slope with the Prism software) for this peptide against ERK1 (Fig. 2). The profiling data for p5-WT against GSK3β showed no inhibition of GSK3β phosphotransferase activity with increasing compound concentration (Fig. 2). At 500 μM concentration of this peptide, the GSK3β phosphotransferase activity was slightly activated by 24% compared to control.

The profiling data for the mutated peptide p5-MT against CDK5/p25 showed strong to potent inhibition of CDK5/p25 phosphotransferase activity with increasing compound concentration (Supplementary Table 7). At 100 μM concentration of this peptide, the CDK5/p25 phosphotransferase activity was inhibited by 46% compared to control. An IC_{50} value of 119 μM was generated (using a graph of log inhibitor versus normalized response with variable slope with the Prism software) for this compound against CDK5/p25 (Fig. 3).

The profiling data for compound p5-MT against CDK5/p35 showed strong to potent inhibition of CDK5/p35 phosphotransferase activity with increasing compound concentration (Supplementary Table 8). At 100 μM concentration of compound p5-MT, the CDK5/p35 phosphotransferase activity was inhibited by 57% compared to control. An IC_{50} value of 83 μM was generated (using a graph of log inhibitor versus normalized response with variable slope with the Prism software) for this compound against CDK5/p35 (Fig. 3).

The profiling data for p5-MT against ERK1 showed weak to moderate inhibition of ERK1 phosphotransferase activity with increasing compound concentrations (Supplementary Table 9). At 500 μM concentration of compound p5-MT, the ERK1 activity was inhibited by 54% compared to control. An IC_{50} value of 425 μM was generated (using a graph of log inhibitor versus normalized response with variable slope with the Prism software) for this compound against ERK1 (Fig. 3). The profiling data for p5-MT against GSK3β showed weak activation of this kinase with increasing compound concentration (Fig. 3). At 500 μM concentration of this peptide, the GSK3β phosphotransferase activity was activated by 40% compared to control.

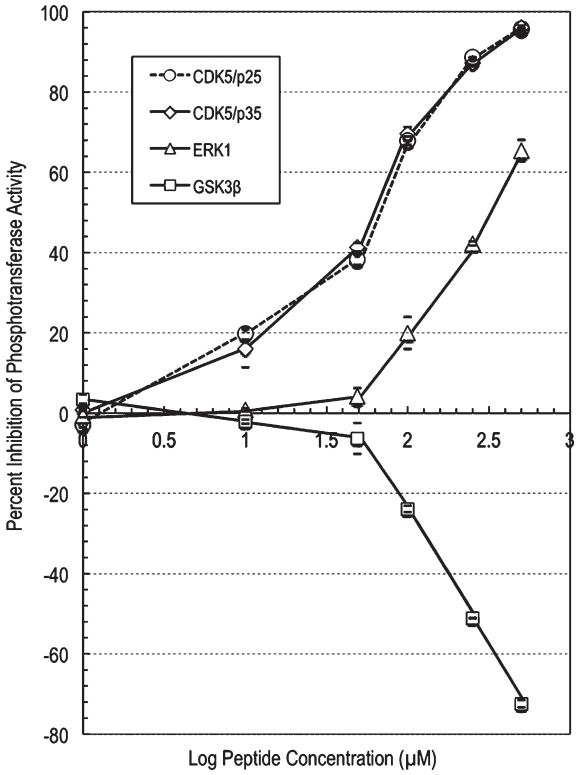

Fig. 2. p5-WT peptide concentration-dependent effects on the phosphotransferase activities of CDK5, ERK1 and GSK3β. IC_{50} determination for compound p5-WT against protein kinases. A graph of log inhibitor versus normalized response with variable slope was generated using the Prism software. The graph showed increased inhibition of the phosphotransferase activities of CDK5 and ERK1, and increased stimulation of GSK3β with increasing compound concentration. The IC_{50} value for compound p5-WT against CDK5/p25 was determined to be 169 μM, the IC_{50} value for compound p5-WT against CDK5/p35 was determined to be 217 μM, and the IC_{50} value for compound p5-WT against ERK1 was determined to be 1322 μM.

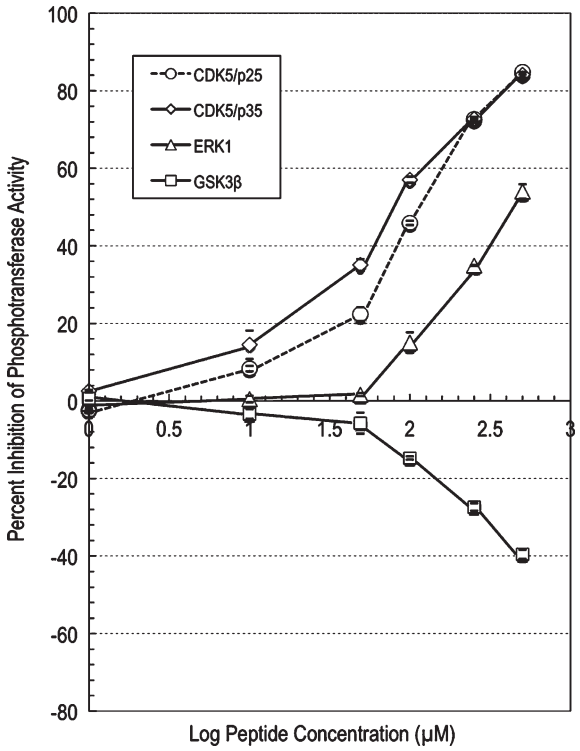

Fig. 3. p5-MT peptide concentration-dependent effects on the phosphotransferase activities of CDK5, ERK1, and GSK3β. IC_{50} determination for compound p5-MT against protein kinases. A graph of log inhibitor versus normalized response with variable slope was generated using the Prism software. The graph showed increased inhibition of the phosphotransferase activities of CDK5 and ERK1, and increased stimulation of GSK3β with increasing compound concentration. The IC_{50} value for compound p5-MT against CDK5/p25 was determined to be 119 μM, the IC_{50} value for compound p5-MT against CDK5/p35 was determined to be 83 μM, and the IC_{50} value for compound p5-MT against ERK1 was determined to be 425 μM.

CONCLUSION

After the production of 78 mimetic peptides with single amino acid substitutions of the p5-WT sequence, the p5-MT peptide with a A24V replacement was determined to be slightly more potent in its inhibition of p25 and p35 stimulation of CDK5 phosphotransferase activity (IC_{50} = 169 to 217 μM for p25 and p35, respectively for p5-WT; IC_{50} = 119 to 83 μM for p25 and p35, respectively for p5-MT). The p5-WT and p5-MT were more extensively tested for their actions on the related proline-directed kinases ERK1 and GSK3β, and ERK1 was modestly inhibited by the p5-MT peptide (IC_{50} = 425 μM) and to a lesser extent by the p5-WT peptide, and the phosphotransferase activity of GSK3-β was weekly stimulated by these peptides.

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SUPPLEMENTARY MATERIAL

The supplementary material is available in the electronic version of this article: http://dx.doi.org/10.3233/JAD-150412.

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